Table IV. Estimated Biopotency of Vitamin A in Stored, Fortified Flours

storage conditions	corn flour	white bread flour
3 months, 40 °C	99	98
3 months, room temp.	98	99
3 months, 45 °C	97	95
12 months[a]		95

[a] Nine months at 45 °C, 3 months at room temperature.

bioactivity of vitamin A remaining in the flour.

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LITERATURE CITED

American Bakers Association Inter-Industry Committee (Prepared by P. M. Ranum), "Recommended Levels and Types of Nutrients to Add to Wheat Flour Under the NAS Expanded Cereal Fortification Proposal", Mimeographed, Pennwalt, Corp., Broadview, IL, 1976.
Ames, S. R., Lehman, R. W., *J. Assoc. Off. Agric. Chem.* **43**, 21 (1960).
Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed, Washington, DC, 1975, 43.008-43.013.
Federal Register **8**, 9170 (1943).
Federal Register **42**, 59513 (1977).
Hepburn, F. N., *Cereal Foods World* **21**, 360 (1976).
Kimble, M. S., *J. Lab. Clin. Med.* **24**, 1055 (1939).
National Academy of Sciences, "Nutrient Requirements of Laboratory Animals", 2nd ed, 1972, p 64.
National Academy of Sciences, Food and Nutrition Board, "Proposed Fortification Policy for Cereal-Grain Products", Washington, DC, 1974, p 36.
Parrish, D. B., *CRC Crit. Rev. Food Sci. Nutr.* **9**, 375 (1977).
Parrish, D. B., Eustace, W. D., Ponte, J. G., *Fed. Proc., Fed. Am. Soc. Exp. Bio.* **37**, 708 (1978).

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Vacuum Thermolysis of 1-Deoxy-1-sarcosino-D-fructose

Thermolysis of the title compound at 140 °C in vacuo yields products that support the earlier suggestion of a preferred decomposition pathway for Amadori compounds substituted with a secondary amino acid. The degradation route proceeds largely through 2,3-enolization of the ketose, with loss of the amino acid, to form a methyl α-dicarbonyl intermediate. The mechanism differs from those of Amadori compounds derived from a secondary amine or primary amino acid in the decomposition of the first-formed intermediate from the 2,3-enolization process. One of the hexose dehydration products derived from this intermediate, 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one, comprises 63% of the isolated distillate. Sarcosine derivatives, a piperazine (31% of the distillate), substituted pyrroles, 2-furfurol, pyrones, and a furanone are other classes of the volatile compounds identified. The findings again demonstrate that the described pyrone and 2,3-dimethyl-4-hydroxy-3(*H*)-furanone are important compounds for the development of browning aromas from sugar–amino acid reaction.

Thermally initiated browning of 1-amino-2-ketoses (Amadori compounds) and the subsequent set of complex reactions that follow characterize in part the genesis of volatile compounds important to food acceptance (Hodge, 1967; Mills et al., 1969, 1970; Reynolds, 1970; Hodge et al., 1972; Mills and Hodge, 1976). These nonvolatile intermediates can be formed by reaction of free and bound amines, amino acids, peptides, or proteins with reducing sugars. The results of earlier model studies, that the decomposition of Amadori compounds had shown a significant difference in decomposition of the 1-substituted 2-ketose when the substituent was changed from a secondary amine to an amino acid (Mills et al., 1970; Mills and Hodge, 1976), are now confirmed with recent reports of the thermolysis of other model 1-amino-1-deoxy-2-ketoses, fructoses containing a 1-valine, -proline, -alanine, or a -4-aminobutyric acid moiety (Shigematsu, 1976; Shigematsu et al., 1977). Because of the later work, the sarcosino-D-fructose study is abbreviated and the conclusions are presented as further support to the initial investigations (Mills et al., 1970; Mills and Hodge, 1976). Our findings demonstrate that the current hexose degradation path agrees with the earlier results (Mills and Hodge, 1976), supporting the concept that 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one, its dehydrogenation product, **13**, and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone are indicators of the browning process in heated or cooked foods (Ledl et al., 1976).

The title Amadori compound was thermally decomposed and the resulting distillate was fractionated by gas–liquid chromatography. The products identified (Table I) were isolated from this distillate except for sarcosine, which was found in the pyrolysis residue, and structural identifications were made spectrometrically. Most assignments were verified by comparison of either gas–liquid chromatography (GLC), infrared (IR), or mass spectral (MS) data, or all of these techniques, with those from synthetic or authentic compounds. As in the past, the low-temperature thermolysis (140 °C) produced fewer types of degradation and rearrangement products compared to those formed at the higher temperatures (Shigematsu, 1976), but these are still important to flavor and aroma development.

Nitrogenous products **1**, **2**, **3**, and **8** are related by class to compounds found in the thermolysis of prolino-D-fructose; their genesis has been described (Mills and Hodge, 1976). Of the nitrogen-containing components, compound **4** represents the only five-carbon hexose fragment, and similar products were not observed in the

Table I. Pyrolysis Products of 1-Deoxy-1-sarcosino-D-fructose

compd	identity	characterization method[a] IR	MS	GLC	SYN	percent distillate[c]	GLC[b] R_t, min A	B	reference
amines									
1	sarcosine				x				
2	3,6-dimethyl-2-5-dioxopiperazine	x	x	x	x	31		47.3	
3	*N*,*N*-dimethylacetamide		x	x	x	Tr		24.5	
4	*N*,*N*-dimethyl-2-furfurylamine		x		x	0.1		30.8	
5	*N*-propionylpyrrole		x			Tr	12.6		Mills and Hodge (1976)
6	*N*-butyrylpyrrole		x			Tr	14.9		Mills and Hodge (1976)
7	unknown (mol wt 149)[d]		x			Tr		63.4	
8	*N*,*N*-dimethyl-5-methyl-2-furfurylamine		x	x	x	Tr		14.5	
non-amines									
9	acetic acid		x		x	5.0	15.5		
10	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	x	x	x	x	Tr	14.8		
11	2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one		x	x	x	63	17.6		
12	2-furfurol		x			Tr	4.2		
13	3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one		x	x	x	Tr	26.7		
14	2-hydroxy-1-methyl-cyclopenten-3-one	x	x	x	x	Tr	14.6		
15	3-hydroxy-2-methyl-4(*H*)-pyran-4-one	x	x	x	x	Tr	21.3		

[a] Spectral data agreed with that obtained for an authentic sample. [b] Columns: (A) 3% 8 BP on 80–100 Chromosorb W, 122 cm × 6 mm programmed 70 °C for 4 min, then 4 °C/min to 225 °C with 32 mL helium flow rate: (B) 3% Silar 5 CP on 80–100 Gas-Chrom Q, 122 cm × 6 mm, programmed at 50 °C and 4 °C/min to 225 °C, helium flow rate 35 mL/min. [c] Represents peak area relative to the total peak area for the products listed. [d] MS, *m/e* (rel %): 149 (100) 104 (5) 95 (12) 93 (8) 79 (4) 78 (8) 76 (4) 69 (6) 68 (9) 67 (7).

earlier thermolyses of 1-secondary amino acid-2-ketoses (Mills and Hodge, 1976; Shigematsu, 1977). Because higher decomposition temperatures cause more fragmentation of the carbohydrate, **4** may be the result of an internal hot spot in the reaction residue. Compounds **5** and **6** indicate that free ammonia is generated from the title 2-ketose, a phenomenon observed in the heating of L-lysine with D-glucose (Bruggemann and Erbersdobler, 1968), allowing ammoniation of other low-molecular-weight fragments that then form the pyrroles. Although a pyrrole was isolated in earlier work (Mills and Hodge, 1976), it was believed to be formed from the decarboxylated amino acid L-proline. Consequently, a difference appears to exist in the formation path of the pyrroles in the two studies. Compound **7** remains unidentified, but its intense molecular ion coupled with the relatively few intense fragments indicate this component is heterocyclic. The aromas of **2**, **4**, **5**, **6**, and **8** were nutty; both **5** and **6** possessed a more burnt characteristic. Products **3** and **7** were minty-aminelike and also had a slight nutty note.

The most abundant, nonnitrogenous component, **11**, is derived from sequential, carbohydrate dehydration reactions, following the 2,3-enolization path proposed earlier and established by the thermolysis of 1-deoxy-1-L-prolino-D-fructose (Mills and Hodge, 1976). This sugar decomposition process occurs in other heated amino acid–carbohydrate systems (Shigematsu et al., 1977) and in heated or cooked foods (Ledl et al., 1976). Components **8**, **10**, **13**, **14**, and **15** also originate from the 2,3-enolization route, but are present only in trace quantities. These findings agree with the previous results obtained from the pyrolysis of proline-D-fructose and indicate that the pyrones, particularly **11** and **13**, and the furanone **10**, which give a powerful burnt-sugar aroma, are indicators of the nonenzymic browning process in food systems that affect the development of the caramel-like aroma. Two exceptions to the components identified in the prolino-D-fructose work are **4** and **12**. Both contain five-carbon, carbohydrate-derived fragments not previously found. These represent a hexose decomposition that is not commonly characteristic of low-temperature pyrolyses.

The results of the sarcosino-D-fructose pyrolysis show that Amadori compounds containing a disubstituted amino group decompose to volatile compounds predominately via a 2,3-enolization of the fructose moiety through formed methyl α-dicarbonyl intermediates. These findings, along with those reported eariler with supportive evidence from other Amadori compound pyrolysis studies, clarify the structures of the compounds formed thermally from sugar–amine reactions and describe pathways by which they are derived. The model studies indicate that these reactions produce characteristic aromas in cooked or heated foods.

EXPERIMENTAL SECTION

General Methods. A Model 1848 Varian Aerograph gas chromatograph equipped with dual-flame detectors was used; one detector was fitted with a variable effluent splitter. An on-column injection technique was employed and stainless-steel columns were used for both analytical and preparative chromatography. Selected effluents were isolated in Teflon tubing that was cooled with dry ice. Authentic reference compounds were synthesized or were available from a previous study (Mills and Hodge, 1976). The IR spectra were obtained with a Perkin-Elmer Model 621 spectrophotometer and from solutions in chloroform. The mass spectra were determined from individual samples at 70 eV or from GLC–MS analysis on a Packard

gas chromatograph coupled to a Nuclide 90G double-focusing mass spectrometer. The ^1H NMR spectra were obtained with a Varian HA-100 instrument from solutions in chloroform-*d* with tetramethylsilane as an internal standard.

1-Deoxy-1-sarcosino-D-fructose. Sarcosine (20 g) and D-glucose (100 g) were reacted in 300 mL of dimethylformamide as previously described (Klemer and Micheel, 1956). The concentrated reaction mixture was dissolved in 1.4 L of water and placed on 454 g of wet packed Dow 50W-4X (H^+) column. The column was eluted with 2 L of water and then with 0.1 M pyridine. The first 3 L of eluate ws discarded and then 15-mL fractions were collected. Those fractions that gave positive ninhydrin and alkaline ferricyanide reactions were combined after analytical chromatography on No. 1 Whatman paper, using 1-butanol/acetic acid/water (4:1:1) as the developing solvent; this showed them to contain only the Amadori compound. After concentration, the syrup was dried by azeotropic distillation of ethanolic solutions. The final residue was taken up in methanol and the solvent was removed; this step was repeated three times. Finally, upon cooling, the product separated from the fourth methanolic solution; mp 82–85 °C (d), yield 32 g.

Anal. Calcd. for $C_9H_{17}O_7N$:CH_3OH: C, 42.40; H, 7.42; N, 4.95. Found: C, 41.87% H, 7.21; N, 4.97.

Pyrolysis. The Amadori compound was decomposed in 10-g amounts at 140 °C and 0.1 torr for 5 h, and the resulting distillate was isolated in a trap cooled in ethanol–dry ice. After warming, the receiver was rinsed with chloroform, and the sample was isolated after drying this solution (sodium sulfate) and removal of the solvent in vacuo at 0 °C; yield, 0.30 g. The oil was then examined by GLC–MS. When required, individual components were isolated by GLC.

3,6-Dimethyl-2,5-dioxopiperazine (Sarcosine Anhydride). Sarcosine (5 g) was heated at its melting point (210 °C) for 3 h. The fused mass was dissolved in water, and the water was then extracted with ethyl acetate. After drying of the organic phase (sodium sulfate) and solvent removal by evaporation, a crystalline precipitate formed; yield 0.360 g, mp 142.5–143 °C. Mass spectrum: *m/e* (rel %) 142 (100), 114 (5), 113 (9), 85 (14), 58 (3), 57 (81), 56 (7), 44 (49), 43 (72).

***N*-*N*-Dimethyl-2-furfurylamine (4) and *N*,*N*-Dimethyl-5-methyl-2-furfurylamine (8).** An excess of thionyl chloride was added to the appropriate 2-furoic acid that was dissolved in benzene. After 6 h of reflux, the solvent and excess thionyl chloride were removed by distillation. The residue was dissolved in chloroform and an excess of *N*,*N*-dimethylamine was added. After stirring for 2 h, the solution was filtered and the solvent removed in vacuo. Proton magnetic resonance, GLS–MS showed the isolate in each preparation to contain only the respective amide (90% yield).

The amide (0.5 g) was reduced using sodium borohydride and pyridine (Kikugawa et al., 1969). Mass spectrum: **4**, *m/e* (rel %) 125 (100), 111 (9), 96 (52), 84 (44), 81 (11), 73 (12), 69 (25), 68 (30). Mass spectrum: **8**, *m/e* (rel %) 139 (100), 124 (8), 110 (26), 95 (6), 82 (30), 81 (10), 57 (16), 55 (27), 44 (55), 47 (88).

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LITERATURE CITED

Bruggemann, J., Erbersdobler, *Z. Lebensm.-Unters. Forsch.* **137**, 137 (1968).
Hodge, J., "Chemistry and Physiology of Flavors", Schultz, H. W., Day, E. A., Libbey, L. M., Eds., Avi, Westport, CT, 1967, Chapter 22.
Hodge J. E., Mills, F. D., Fisher, B. E., *Cereal Sci. Today* **17**, 34 (1972).
Kikugawa, Y., Ikegami, S., Yamada, S., *Chem. Pharm. Bull. (Tokyo)* **17**, 98 (1969); CA 70, 87491C.
Klemer, A., Micheel, F., *Chem. Ber.* **89**, 1242 (1956).
Ledl, F., Schnell, W., Severin, T., *Z. Lebensm.-Unters. Forsch.* **160**, 367 (1976).
Mills, F. D., Baker, B. G., Hodge, J. E., *J. Agric. Food Chem.* **17**, 723 (1969).
Mills, F. D., Baker, B. G., Hodge, J. E., *Carbohydr. Res.* **15**, 205 (1970).
Mills, F. D., Hodge, J. E., *Carbohydr. Res.* **51**, 9 (1976).
Reynolds, T. M., *Food Technol. Aust.* **22**, 610 (1970).
Shigematsu, H., *Nippon Sembai Kosha, Chuo Kenkyiyo Kenkyo Nokoy* **11B**, 119 (1976).
Shigematsu, H., Shibata, S., Kurata, T., Kato, H., Fujimaki, M., *Agric. Biol. Chem. (Jpn)* **41**, 2377 (1977).

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Peanut Leaf Extract. Chemical Composition and Protein Characterization

A peanut leaf extract obtained from fresh leaves contained 42.2% carbohydrate, 25.4% ash, 18.0% protein, 8.8% fat, 4.6% moisture, and 0.8% fiber on a dry basis. Over 65% of the fatty acids comprised linolenic, palmitic, and linoleic acids; the concentration of oleic acid was higher than in most leaf tissues. Amino acid composition of protein in peanut leaf extract was comparable to that of similar extracts derived from other leaf tissues lacking in sulfur-containing amino acids. Calculations from amino acid data showed that the estimated biological value of the extracted leaf protein was less than that of casein or egg. Serological and electrophoretic analyses of protein in the peanut leaf extract showed several associative species, some of which exhibited antigenic identity but varied in electrophoretic mobility and molecular size.

In recent years, several investigators have emphasized the unconventional use in human nutrition of protein from various leaves (Betschart and Kinsella, 1973; Edwards et al., 1975, Kohler et al., 1977; Pirie, 1971). Because of the tremendous worldwide supply of leafy crops produced each year, it seems logical that much of the protein therefrom